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MICROBIAL DETERIORATION OF HYDROCARBON FUELS FROM OIL SHALE, CO--ETC(U)
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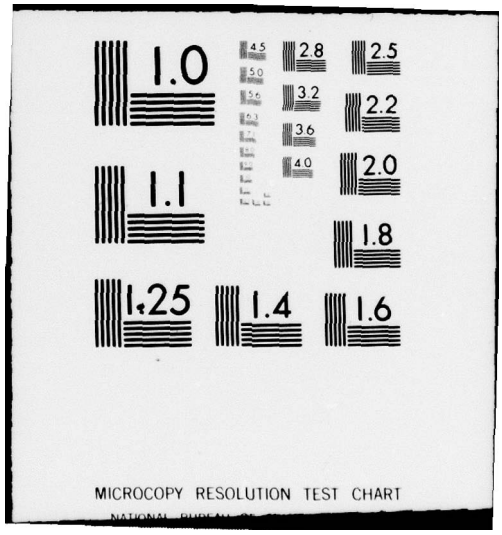
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NRL Memorandum Report 4060

12 LEVEL III

Microbial Deterioration of Hydrocarbon Fuels from Oil Shale, Coal, and Petroleum I. Exploratory Experiments

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Petroleum	Cladosporium resinae	Microbial deterioration
JP-5	Candida sp.	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

As part of the Navy's program on alternative sources of hydrocarbon fuel, the susceptibility to microbial deterioration of JP-5 derived from oil shale and coal (referred to as synthetic fuels) was investigated and compared with that of petroleum JP-5. Six fungi, including three strains of Cladosporium resinae, a yeast (Candida) and a bacterium (Pseudomonas) which normally grow well in association with petroleum JP-5 were used as test organisms in two-phase systems containing fuel/aqueous media. Most of the test organisms were inhibited to various extents in the presence

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of the synthetic fuels. An exception was a Fusarium species (fungus) which grew equally well under all three fuels. In mixtures of 75% petroleum and 25% synthetic fuels, microbial growth was generally equivalent to that in 100% petroleum JP-5. A search was made among samples of soil, creosoted wood and tree resins for microorganisms that could thrive in the presence of synthetic fuels. This endeavor produced a strain of C. resinae that grew as well with oil shale JP-5 as with petroleum JP-5. These exploratory experiments indicate that microorganisms adapted to growth with conventional petroleum fuel tend to be inhibited by synthetic fuels, but that organisms probably exist in nature which can readily adapt to and grow in the presence of synthetic fuels.

CONTENTS

MICROBIAL DETERIORATION OF HYDROCARBON FUELS FROM OIL SHALE, COAL AND PETROLEUM. I. EXPLORATORY EXPERIMENTS

INTRODUCTION

The decreasing availability of secure sources of crude petroleum and the almost total dependence of military ships, aircraft and vehicles on liquid hydrocarbon fuels has forced an evaluation of fuels derived from alternate sources under domestic control. The Naval Air Systems Command has the task of evaluating jet aircraft fuels from sources such as coal and oil shale as part of an Energy Conversion Synthetic Fuels Program (3).

Studies of the chemical and physical properties of kerosene-type fuels from different crude sources refined in different ways are in progress (1,9,10). Another property of fuels from alternate sources not yet evaluated is their susceptibility to microbial contamination. Such an investigation appears necessary in view of the occurrence of problems in the past from microbial growth with conventional fuels (8). A relatively high content of nitrogen compounds in both shale and coal derived fuels and a high n-alkane content of shale fuels could well be factors favoring microbial growth (7,8,9). Potential problems in the use of new fuels should obviously be recognized as early as possible so that appropriate countermeasures can be developed before the fuels become widely used.

This report describes initial evaluations of the susceptibility to microbial contamination of JP-5 type fuels derived from crudes produced from oil shale by the Paraho process (4) and from coal by the Char Oil Energy Development process (COED) (4). Comparisons were made with conventional JP-5 from petroleum. Experimental work was carried out in small, two-phase test units consisting of fuel overlying an equal volume of an aqueous phase. Two different types of aqueous phases were used — dilute mineral salts solution and seawater. The first simulated field conditions where water of condensation or fresh water has collected in storage facilities, and the second simulated conditions in shipboard seawater compensated or ballasted fuel tanks. In addition to tests with neat fuels, mixtures of the synthetic fuels with conventional JP-5 were used, since such mixtures are likely to be used in actual practice during a transition period.

The microbial inocula in the initial investigations came from contaminated conventional diesel fuels or jet fuels. However, it was recognized that isolates which grow well with conventional fuels might

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not necessarily thrive with fuels from non-petroleum sources and vice versa. Consequently, other microbial strains, especially of the fuel fungus, Cladosporium resinae, were sought in a variety of natural habitats. The results of this survey are also included in this report.

MATERIALS AND METHODS

Fuels

The petroleum base fuel was designated Jet-A. It contained no additives and was received in January 1976 from Naval Air Propulsion Test Center, Trenton, N.J.

Coal derived liquid crude oil produced from Western Kentucky Coal was subsequently processed by Sun Oil Co. using high pressure catalytic hydrogenation to yield a fuel that met most of the requirements for JP-5.

The oil shale product was from crude produced by the Paraho process in Northwestern Colorado which was subsequently refined into military fuels. The fuel employed here did not meet JP-5 specifications for gum content, thermal stability and freezing point (10). Both synthetic fuels were received at NRL in January of 1978 and were subsequently stored at 4°C.

A summary of selected properties of the fuels is presented in Table 1.

Water Phase

Simulated fresh water media consisted exclusively of mineral salts and followed the formulation of Bushnell and Haas (2). This solution had a pH of about 6.5 after sterilization. Following a finding of Parbery (8) that some strains of Cladosporium resinae do not grow well in Bushnell-Haas medium containing the normal amount of K_2HPO_4, tests were also run omitting this constituent. The pH in this case was about 5.0.

All of the seawater used in these tests was obtained about 30 km east of Ocean City, MD. It had been aged for over a year in the dark at 4°C before use and had a pH of between 7.85 and 8.0. In some test units the seawater was used alone; in others the seawater had 1 g/l of KH_2PO_4 added to it which brought the pH down to 5.6 - 5.8; in still other test units the seawater had additions of 0.05% peptone (Difco) and 0.05% yeast extract (Difco) and the pH was about 7.8.

Inocula

Four different strains of C. resinae were chosen for this study along with another fungus and a yeast commonly found associated with fuel/water systems in the field. A common marine bacterium was also included. The identification and sources of these organisms are as follows:

Cladosporium resinae (strain DK) isolated from a JP-5 storage tank at a Naval Air Station, Lemoore, CA.

Cladosporium resinae f. avellaneum isolated from a sample of water with a skim of oil on the surface which had collected in an exposed boiler room of a naval ship in the process of being scrapped at Curtis Bay, MD.

Cladosporium resinae (strain P-1) isolated from sludge in the No. 2 purifier on the USS PETERSON.

Cladosporium resinae (strain P-4) isolated from a sample of mixed diesel fuel and seawater taken through a dewatering tube in a fuel storage tank on the USS PETERSON.

Candida sp. isolated from the same source as the C. resinae f. avellaneum above.

Fusarium sp. isolated from the same source as C. resinae (P-4) above.

Pseudomonas marina from the American Type Culture Collection (ATCC 27129).

Also used as inoculum was a combination of the Candida sp. and the C. resinae f. avellaneum. These two organisms were isolated from the same source and, with the thought that synergism might be involved, they were also tested together.

For inoculation the fungi and the yeast were grown on potato-dextrose agar (Difco) slants (in 20 x 125 mm test tubes) with the addition of 0.5% yeast extract (Difco). The P. marina was similarly grown on marine agar 2216 (Difco) slants. Stock suspensions of organisms were prepared by dispersing surface growth on a slant in 10 ml of a solution of 0.05% "Tween 80" in distilled water. The viable count in these suspensions was 26 to 56 x 10^6 per ml for the yeast and fungi and 10^6 for the P. marina. Where mixed inocula were used the viable count was about 23 x 10^6/ml for Candida and 17 x 10^6/ml for C. resinae.

Test Units

The test units were 250 ml Erlenmeyer flasks. Fifty ml of the aqueous phase were dispensed into each flask and autoclaved for 20 min at 120°C. Fifty ml of fuel (unsterilized) were then added followed by 0.5 ml of the microbial stock suspension. The flasks were plugged with cotton, loosely covered with aluminum foil and incubated in the dark at room temperature (22° - 25°C). Control units with no microbial inoculum were included for every fuel/aqueous medium combination.

Monitoring Microbial Growth

The test units inoculated with fungi and yeast were visually inspected for growth at 1, 3, 7, 13 and 26 weeks. The rating system is given in Table 3. Comparisons were made with uninoculated control units to permit allowances for insoluble materials in the water or at

the oil/water interface not due to microbial growth. The presence of growing organisms introduced in the unsterilized fuel could also be detected by examination of the control units. After 13 weeks and 26 weeks, viability studies were made on those test units inoculated with yeast or fungi and showing doubtful or no growth (rated ± or -) by spreading 0.1 ml of the water and fuel from the interface in the test units on the appropriate potato-dextrose agar containing 0.5% yeast extract. Those units with clearly established growth (rated + or more) were not plated because viability was assured from past experience.

The bacterium, P. marina, did not grow sufficiently in the test units to allow a visual assessment. Instead 0.1 ml of the aqueous phase was removed at 1, 3, 7, 13 and 26 weeks and spread on marine agar 2216 plates. Ratings of the growth on these plates were recorded.

At 26 weeks pH measurements of the aqueous phase were made in all test units.

Additional Field Collections

In an effort to determine if microorganisms exist which can more readily adapt to growing in the presence of coal and oil shale derived fuels than those associated with conventional fuel, a number of field collections were made from sources which have been considered selective for microorganisms able to grow in the presence of hydrocarbons, especially C. resinae (8). Samples of creosote, creosoted wood and oil saturated materials, including soils, were collected in the Baltimore Harbor area and along the Potomac River south of Washington, DC. A group of tree resins from California was also collected (8). Locations and descriptions of all samples are given in Table 2.

Small amounts of the samples were placed in 20 x 125 mm culture tubes containing the sterile aqueous media, either mineral salts solution or 10% seawater with 0.05% peptone and 0.05% yeast extract. The water phases were overlaid with one of the three fuels being studied. Cultures were made on the same day as the collection except in the case of those from California where 12-20 days elapsed before culturing. These enrichment cultures were observed for up to one year. Selected tubes showing growth during this period were subcultured for further study.

RESULTS AND DISCUSSION

Tables 4-11 show the growth, survival and final aqueous pH for fungi and yeast in the different fuel/aqueous media test systems used and Table 3 defines the abbreviations and rating systems employed in these tables. Table 12 shows results with a bacterium.

Fuel Effects

Tables 4-7 show results with test units inoculated with pure cultures of different strains of C. resinae from contaminated petroleum

base fuel systems. These organisms did not germinate, grow or survive as well under fuel from coal or oil shale as under petroleum JP-5. However, with a mixture of 75% JP-5 from petroleum and 25% of either of the synthetic fuels there was little difference in growth and survival from the systems with 100% petroleum fuel. Apparently the constituent which is inhibitory to C. resinae in 100% shale and coal derived fuels can be readily rendered innocuous by dilution.

The growth and survival of Candida sp. were also markedly inhibited even in favorable aqueous media by the two synthetic fuels but not by a 25% mixture of coal fuel in petroleum (Table 8). A 25% mixture of shale oil fuel in conventional fuel was clearly still inhibitory in all seawater media though not with mineral salts.

The mixed culture of Candida and C. resinae f. avellaneum (Table 9) did not grow or survive in the synthetic fuels significantly better than either organism alone. In the synthetic-petroleum fuel mixtures growth of the two organisms in 26 weeks was comparable to that in 100% petroleum fuel, however, the early growth rate was slower. In those cases where prolific growth occurred in these test units, it should be emphasized that C. resinae as well as Candida was involved. This was established by microscopic examination and by the fact that the interfacial mat was coherent and tough when C. resinae was present but not when Candida was alone. In view of this a comparative examination of the data from Table 9 with that for the pure culture of C. resinae f. avellaneum of Table 5 shows the growth of the latter was aided by the presence of the yeast. Indeed with the 25% shale oil-SW + PY system, growth of the mixed inoculum was markedly better than for either organism alone (cf. Table 8). In view of the common coexistence of Cladosporium and a yeast in contaminated fuel systems it seems possible that synergism may be an important factor.

Fusarium grew well under all fuels in a seawater medium to which a little nutrient (peptone-yeast) has been added (Table 10). In seawater without added nutrient, growth of Fusarium was considerably reduced but only with the coal derived fuel was there indication of poorer growth and survival than with petroleum JP-5.

P. marina survival was significantly inhibited by the synthetic fuels, particularly oil shale fuel, as compared with petroleum fuel (Table 11).

Aqueous Media Effects

All four of the C. resinae strains grew more readily in the fresh water media (mineral salts) than in seawater (Tables 4-7). Two strains of C. resinae, P-1 and P-4, (Tables 6 and 7) survived better in seawater than the DK strain (Table 4). This may be an indication of adaptation or of strain selection in a salt water medium, since P-1 and P-4 were from seawater displaced fuel tanks and strain DK was from a land based storage tank containing essentially fresh water. Hendey (5) has also found that concentrations of seawater in distilled water

above 30% retarded the growth of a C. resinae strain he was using. The addition of nutrient (PY) to seawater did not improve growth or survival of C. resinae (Tables 4 and 5) but KH_2PO_4 did (Tables 4-7). It should be noted that addition of KH_2PO_4 to seawater lowered the pH from about 8.0 to about 5.7. Thus the improved response brought about by KH_2PO_4 may be due as well to a lower pH as to an increase in phosphate or potassium.

Growth of Candida was also inhibited in the presence of seawater but the addition of a little organic nutrient (PY) allowed growth comparable to that in mineral salts (with petroleum fuel) (Table 8). The addition of KH_2PO_4 stimulated growth of Candida in seawater to a much smaller extent than nutrient (in contrast to the response of C. resinae). The growth stimulus offered by traces of nutrient has a similarity to Kuo's experience with a yeast from a contaminated fuel system which did not grow in tanks containing distilled water, tap water or tank bottom water but survived and multiplied when dust was added to the water (6).

Generally the conditions which were favorable for growth of yeast alone were favorable for growth of C. resinae with the yeast (Table 9). In the case of the oil shale-petroleum fuel mixture with seawater containing peptone-yeast extract, growth of the two organisms together was considerably better than with either alone (cf. Tables 5, 8 and 9). Thus under some conditions there may be an interaction favoring the mutual growth of these two organisms.

Prolific growth in Candida led to pH reductions to as low as 2.3 in mineral salts media of limited buffer capacity (without K_2HPO_4) and to 3.4 in seawater with added nutrient. The C. resinae strains used here have a lesser capacity to lower media pH. While nothing is known about the pH limits for growth of these particular organisms it is possible that seawater inhibits Cladosporium because of the high pH and that the improved growth noted in the presence of Candida was due to the greater ability of the latter to lower the pH to a more suitable range. This might explain the frequent occurrence of these two species in field samples containing seawater. Appropriate experiments are in progress to test this hypothesis.

The growth of Fusarium was greatly stimulated by the addition of organic nutrient (PY) and, unlike Candida and Cladosporium, produced no reduction in media pH; indeed, the pH tended to rise with growth.

Additional Microbial Collections

Table 12 summarizes the results of efforts to find microorganisms on selected materials collected in the field which were able to grow in the presence of synthetic fuels. In general, growth was observed more often with the petroleum base fuels than with the two synthetic fuels. With the tree resin collections no growth with coal derived fuel was ever observed. A possible speculative explanation for these findings is that the petroleum JP-5 is related to a natural product with a microbial origin while the synthetic fuels, as the name suggests,

comes from a non-petroleum source and the organic base, kerogen, must undergo considerable covalent bond breakage and molecular rearrangement during processing in order to become acceptable as a fuel. These fuels may well contain products for which no adaptation in the natural microbial world has been possible. Nevertheless, several samples exhibited prolific growth with synthetic fuels particularly at the water/fuel interface. Figure 1 shows typical examples under coal JP-5. Subcultures from interfacial growth in these cultures onto agar plates followed by reinoculation into mineral salts media under synthetic fuels led in some cases to pure microbial cultures which grew in two-phase mineral salts/fuel systems. Figure 2 shows one such example of a C. resinae strain which grew well under fuel from oil shale and petroleum but not from coal.

The isolation procedure just described did not always yield pure cultures which would grow under the synthetic fuels in spite of the prolific growth observed in the original enrichment cultures. This may be due to synergism among the different organisms originally present or to the presence of nutrient material carried along with the original field material which sustained growth initially but was insufficient after subculturing.

ACKNOWLEDGMENT

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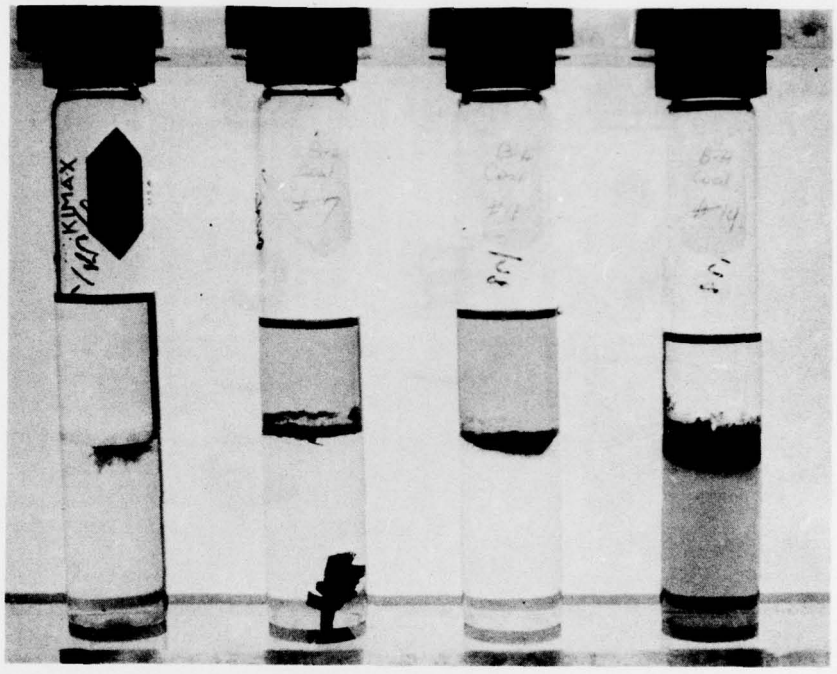

Fig. 1 — Examples of microbial growth at the interface of two-phase test tube enrichment cultures consisting of a mineral salts solution overlayed with JP-5 derived from coal. From the left, samples 6-7/26/78, 7-7/26/78, 11-7/26/78 and 14-7/26/78 (Table 12) are shown.

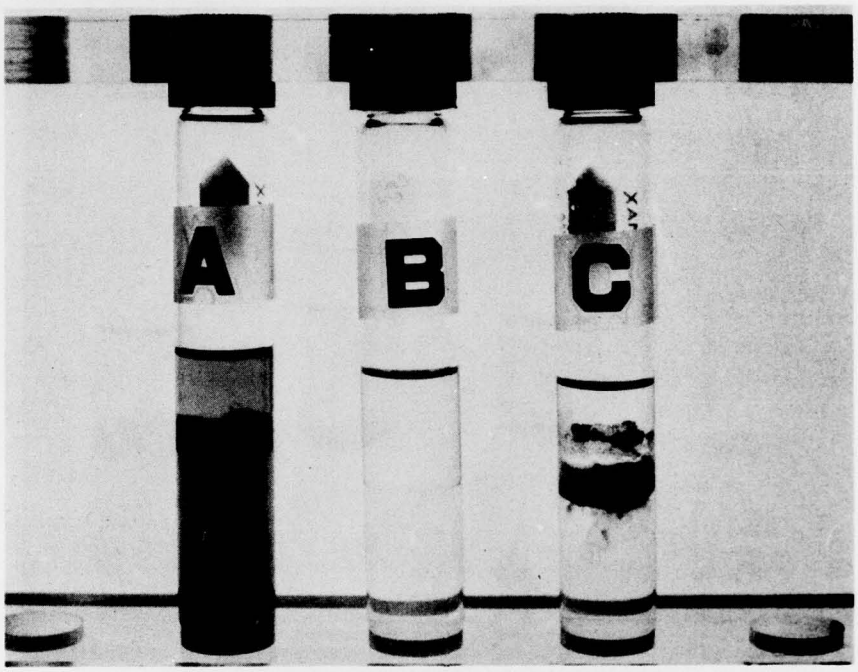

Fig. 2 — A C. resinae strain, isolated from sample 5-4/21/78 (Table 12) showing good growth (4+) in mineral salts overlaid with (A) oil shale JP-5 and (C) petroleum JP-5, but no growth on (B) coal JP-5.

Table 1. Chemical compositions of JP-5-type fuels used in microbial investigations[a].

JP-5 source	Nitrogen ppm	Sulfur wt.-%	Aromatics vol.-%	n-Alkanes vol.-%	Hydrogen wt.-%	Oxygen wt.-%
Oil shale (Paraho-I)	860	0.05	26.0	36.8	13.74	0.28
Coal (COED-5)	37	0.03	24.1	5.4	12.81	0.00
Petroleum (typical)	3	0.11	16.2	15.9	13.7	0.42

[a]From Brinkman, D.W. et al. (1) and Solash, J. et al. (9).

Table 2. Location and description of sources of inocula used in search for microorganisms that will grow in the presence of synthetic fuel.

Sample	Location	Source description
	Baltimore Harbor Area	
1-4/21/78	Ft. Smallwood Park	Scrapings from creosote pilings washed up on beach.
2-4/21/78	Ft. Smallwood Park	Tarred rope on beach
3-4/21/78	Ft. Smallwood Park	Scrapings from pilings on pier
4-4/21/78	Ft. Armistead Park	Scrapings from old pilings beneath new pier.
5-4/21/78	Ft. Armistead Park	Sample of creosoted wood cut from new construction timber above water.
6-4/21/78	Ft. McHenry	Wood from a creosoted piling washed up on the rocks.
7-4/21/78	U.S. Army Corps of Engineers, Ft. McHenry	Scrapings from 2-year old pier; creosote still bleeding into surrounding water.
8-4/21/78	U.S. Army Corps of Engineers, Ft. McHenry	Scrapings from old pier next to new pier.

Table 2. (Cont'd.)

Sample	Location	Source description
	Potomac River area south of Washington	
1-7/26/78	Captain's Cove Marina	Pier soaked with oil spill
2-7/26/78	Captain's Cove Marina	Creosoted piling under pier
3-7/26/78	Captain's Cove Marina	Soil next to creosoted retaining wall.
4-7/26/78	Captain's Cove Marina	Lumps of creosote or tar on top of wood retaining wall.
5-7/26/78	Ft. Washington National Park	Creosote or tar dumped near the Potomac River's edge.
6-7/26/78	Tantallon Marina	Soil where oil has been dumped around a metal pole to prevent grass growth.
7-7/26/78	Alexandria Marina	Sample of piling on old pier
8-7/26/78	Alexandria Marina	Sample of wooden jetty off of old pier.
9-7/26/78	Alexandria Potomac Power Co.	Outside of gate; bits of coal and soil between fence and railway.
10-7/26/78	Alexandria Potomac Power Co.	Bits of coal and soil between fence and railway.
11-7/26/78	Alexandria Potomac Power Co.	New creosoted railroad ties between fence and railway.
12-7/26/78	Alexandria Potomac Power Co.	Soil from around the above railroad ties.
13-7/26/78	NRL Pier	Creosoted wood inside of fence
14-7/26/78	NRL Pier	Soil below creosoted wood inside of fence.

Table 2. (Cont'd.)

Sample	Location	Source description
	Tree resins from California	
M1-9/2/78	Monterey	Monterey Pine
M2-9/2/78	Monterey	Coast Redwood (Sequoia semper virens)
M3-9/2/78	Monterey	Eucalyptus globulus (Blue gum)
M4-9/2/78	Monterey	Monterey Cypress (Cupressus macrocarpa)
M5-9/2/78	Monterey	Araucaria
M6-9/2/78	Monterey (Jacks Peak)	Monterey Pine
M7-9/3/78	Carmel	Eucalyptus
M8-9/3/78	Monterey (Jacks Peak)	Pine (Pinus radiata)
01-9/10/78	Oxnard	Eucalyptus
02-9/10/78	Oxnard	Eucalyptus
03-9/10/78	Oxnard	Black paint on tree wound

Table 3. Key to (i) the rating system for growth in two-phase fuel/water systems (Tables 4-9 and 12); (ii) the rating system for viability (Tables 4-10); and (iii) the abbreviations used in Tables 4-12.

Symbol	Description
Growth	
-	No germination, growth or mat formation detected.
±	Germination and minute amount of growth detected; no mat formation.
+	Slight growth like "peach fuzz" on the underside of the interface.
2+	Mat formation over 1/3 of interface.
3+	Mat formation over 2/3 of interface.
4+	Mat formation over entire interface.
5+	Mat formation over entire interface with a thickness of at least 0.5 cm.
Viability	
v	Viable
nv	Not viable
nd	Not done
Abbreviations	
SW	Seawater
SW + KH_2PO_4	Seawater + 1 g/l KH_2PO_4
SW + PY	Seawater + 0.05% peptone and 0.05% yeast extract
MS	Mineral salts. Bushnell-Haas (2).
MS - K_2HPO_4	Mineral salts without K_2HPO_4

Table 4. Growth of C. resinae DK in fuel/water test units; viability on subculture; and final pH of water phase[a].

JP-5 source	Water phase	Ratings of growth after incubation (weeks) 1	3	7	13	26	Viability after 13 weeks	26 weeks	pH at 26 weeks
Petroleum	MS	2+	3+	4+	4+	5+	nd	nd	4.3
	SW	-	-	-	-	-	nv	nv	7.9/7.7
	SW + KH_2PO_4	±	±	±	±	+	v	nd	5.4/5.5
	SW + PY	±	±	±	±	±	v	v	7.6/7.9
Coal	MS	-	-	±	±	+	v	nd	6.4/6.4
	SW	-	-	-	-	-	nv	nv	8.0/7.9
	SW + KH_2PO_4	-	-	±	±	-	v	nv	5.7/5.6
	SW + PY	-	-	-	-	-	nv	nv	7.7/7.9
Oil shale	MS	±	±	±	±	±	v	v	6.9/6.9
	SW	-	-	-	-	-	nv	nv	7.9/7.9
	SW + KH_2PO_4	-	-	-	-	-	v	v	6.5/6.5
	SW + PY	-	-	-	-	-	nv	nv	7.85/7.85
75% Petroleum 25% Coal	MS	2+	3+	3+	4+	4+	nd	nd	4.3/4.3
	SW	-	-	-	-	-	nv	nv	8.0/8.0
	SW + KH_2PO_4	±	±	±	+	1½+	nd	nd	5.6/5.6
	SW + PY	-	-	-	-	-	nv	nv	7.95/7.9
75% Petroleum 25% Oil shale	MS	+	2+	3+	4+	4+	nd	nd	4.6/4.8
	SW	-	-	-	-	-	nv	nv	8.1/7.9
	SW + KH_2PO_4	±	±	±	+	1½+	nd	nd	6.0/5.8
	SW + PY	-	-	-	-	-	nv	nv	7.9/7.75

[a]See Table 3 for definition of symbols

Table 5. Growth of C. resinae f. avellaneum in fuel/water test units; viability on subculture; and final pH of water phase[a].

JP-5 source	Water phase	Ratings of growth after incubation (weeks)					Viability after		pH at
		1	3	7	13	26	13 weeks	26 weeks	26 weeks
Petroleum	MS - K_2HPO_4	±	±	±	±	±	v	v	4.9/4.5
	SW	-	-	-	-	-	v	nv	8.0/8.0
	SW + KH_2PO_4	-	-	-	-	-	v	v	5.5/5.6
	SW + PY	-	-	-	-	-	nv	nv	8.05/7.9
Coal	MS - K_2HPO_4	-	-	-	-	-	v	v	4.9/4.6
	SW	-	-	-	-	-	nv	nv	8.0/8.0
	SW + KH_2PO_4	-	-	-	-	-	v	nv	5.45/5.65
	SW + PY	-	-	-	-	-	nv	nv	7.9/7.9
Oil shale	MS - K_2HPO_4	-	-	-	-	-	v	v	6.6/6.6
	SW	-	-	-	-	-	nv	nv	5.8/7.7
	SW + KH_2PO_4	-	-	-	-	-	nv	nv	6.5/6.5
	SW + PY	-	-	-	-	-	nv	nv	7.9/7.9
75%	MS - K_2HPO_4	-	±	±	±	±	v	v	4.6/4.3
Petroleum	SW	-	-	-	-	-	v	nv	7.6/7.9
25%	SW + KH_2PO_4	-	-	-	-	-	v	v	5.6/5.7
Coal	SW + PY	-	-	-	-	-	v	v	7.9/8.0
75%	MS - K_2HPO_4	-	-	-	-	±	v	v	6.2/6.1
Petroleum	SW	-	-	-	-	-	nv	nv	8.0/7.9
25%	SW + KH_2PO_4	-	-	-	-	-	v	v	6.0/6.0
Oil shale	SW + PY	-	-	-	-	-	nv	nv	7.9/7.9

[a]See Table 3 for definition of symbols.

Table 6. Growth of C. resinae P1 in fuel/water test units; viability on subculture; and final pH of water phase[a].

JP-5 source	Water phase	Ratings of growth after incubation (weeks) 1	3	7	13	26	Viability after 13 weeks	26 weeks	pH at 26 weeks
Petroleum	SW	-	-	-	-	-	v	v	8.0/8.0
	SW + KH_2PO_4	±	±	±	±	-	v	v	5.5/5.5
Coal	SW	-	-	-	-	-	nv	nv	7.9/7.9
	SW + KH_2PO_4	-	-	-	-	-	v	nv	5.6/5.6
Oil shale	SW	-	-	-	-	-	v	nv	7.45/7.8
	SW + KH_2PO_4	-	-	-	-	-	v	nv	6.5/6.5
75% Petroleum 25% Coal	SW	-	-	-	-	-	v[b]	v[b]	7.9/8.0
	SW + KH_2PO_4	±	±	±	±	-	v	v	5.7/5.7
75% Petroleum 25% Oil shale	SW	-	-	-	-	-	v[b]	nv	7.85/7.7
	SW + KH_2PO_4	±	±	±	±	-	v	v	6.1/5.95

[a]See Table 3 for definition of symbols.

[b]Very low viability.

Table 7. Growth of C. resinae P4 in fuel/water test units; viability on subculture; and final pH of water phase[a].

JP-5 source	Water phase	Ratings of growth after incubation (weeks) 1	3	7	13	26	Viability after 13 weeks	26 weeks	pH at 26 weeks
Petroleum	SW	-	-	-	-	-	v	v[b]	7.85/7.8
	SW + KH_2PO_4	±	±	±	±	±	v	v	5.55/5.6
Coal	SW	-	-	-	-	-	nv	nv	8.0/8.0
	SW + KH_2PO_4	-	-	-	-	-	nv	nv	5.6/5.6
Oil shale	SW	-	-	-	-	-	v	nv	7.9/7.7
	SW + KH_2PO_4	-	-	-	-	-	v	nv	6.5/6.4
75% Petroleum 25% Coal	SW	-	-	-	-	-	v	nv	7.9/7.85
	SW + KH_2PO_4	±	±	±	±	±	v	v	5.5/5.5
75% Petroleum 25% Coal	SW	-	-	-	-	-	v[b]	nv	8.0/7.95
	SW + KH_2PO_4	±	±	±	±	-	v	v	6.0/6.0

[a] See Table 3 for definition of symbols.

[b] Very low viability.

Table 8. Growth of Candida sp. in fuel/water test units; viability on subculture; and final pH of water phase[a].

JP-5 source	Water phase	Ratings of growth after incubation (weeks) 1	3	7	13	26	Viability after 13 weeks	26 weeks	pH at 26 weeks
Petroleum	MS - K_2HPO_4	+	+	2+	3+	4+	nd	nd	2.3/2.3
	SW	±	±	±	±	±	v	v	7.85/7.65
	SW + KH_2PO_4	±	+	+	+	2+	nd	nd	5.4/5.4
	SW + PY	2+	3+	3+	4+	4+	nd	nd	3.4/3.35
Coal	MS - K_2HPO_4	-	±	±	+	2+	nd	nd	3.3/3.1
	SW	-	±	±	-	-	nv	nv	7.95/7.9
	SW + KH_2PO_4	-	-	-	-	-	v	nv	5.7/5.6
	SW + PY	-	±	±	±	-	v	nv	8.0/8.0
Oil shale	MS - K_2HPO_4	-	-	-	±	+	v	v	6.7/6.4
	SW	-	±	±	-	-	nv	nv	8.0/7.9
	SW + KH_2PO_4	-	-	-	-	-	v	v	6.6/6.5
	SW + PY	-	-	-	-	-	v	v	8.0/8.0
75%	MS - K_2HPO_4	2+[b]	2+[b]	3+[b]	4+[b]	4+[b]	nd	nd	2.6/2.6
Petroleum	SW	-	±	±	±	-	v	v	8.0/8.0
25%	SW + KH_2PO_4	±	+	+	2+	2+	nd	nd	5.5/5.4
Coal	SW + PY	+	2+	3+	4+	4+	nd	nd	3.4/3.4
75%	MS - K_2HPO_4	+	+	2+	3+	4+	nd	nd	4.2/4.1
Petroleum	SW	-	-	±	±	-	v	v	8.0/8.05
25%	SW + KH_2PO_4	-	±	±	±	-	v	v	6.0/6.0
Oil shale	SW + PY	±	±	±	±	-	v	v	8.0/8.0

[a] See Table 3 for definition of symbols

[b] Contaminated with a wild C. resinae.

Table 9. Growth of a combination of C. resinae f. avellaneum and Candida sp. in fuel/water test units; viability on subculture; and final pH of water phase[a].

JP-5 source	Water phase	Ratings of growth after incubation (weeks) 1	3	7	13	26	Viability after 13 weeks	26 weeks	pH at 26 weeks
Petroleum	MS - K_2HPO_4	2+	3+	4+	4+	4+	nd	nd	2.2/1.9
	SW	-	+	+	+	+	nd	nd	7.65/7.9
	SW + KH_2PO_4	±	±	+	+	+	nd	nd	5.6/5.5
	SW + PY	2+	3+	4+	4+	4+	nd	nd	3.4/3.6
Coal	MS - K_2HPO_4	-	-	-	+	2+	nd	nd	2.3/2.2
	SW	-	-	-	-	-	v[b]	nv	7.9/7.7
	SW + KH_2PO_4	-	-	-	-	-	v[b]	nv	5.6/5.7
	SW + PY	-	±	+	+	+	nd	nd	8.2/8.0
Oil shale	MW - K_2HPO_4	-	-	-	-	+[c]	v	v	6.7/6.75
	SW	-	-	-	-	-	v[b]	nv	8.0/8.0
	SW + KH_2PO_4	-	-	-	-	-	v[b]	v[b]	6.5/6.5
	SW + PY	-	-	-	-	-	v[b]	v[b]	8.0/7.9
75% Petroleum 25% Coal	MS - K_2HPO_4	2+	2+	3+	3+	3+	nd	nd	2.2/2.3
	SW	-	±	+	+	+	nd	nd	8.1/8.0
	SW + KH_2PO_4	±	+	+	+	+	nd	nd	5.6/5.6
	SW + PY	+	2+	4+	4+	4+	nd	nd	3.5/3.5
75% Petroleum 25% Oil shale	MS - K_2HPO_4	+	2+	2+	4+	4+	nd	nd	3.4/3.8
	SW	-	-	-	±	-	v[d]	v[d]	7.7/7.9
	SW + KH_2PO_4	±	±	+	+	+	nd	nd	6.0/6.0
	SW + PY	-	±	±	2+	4+	nd	nd	5.2/4.9

[a] See Table 3 for definition of symbols.
[b] Very low viability of Candida sp. only.
[c] One of the duplicates shows growth and one no growth.
[d] Candida sp. viable.

Table 10. Growth of Fusarium sp. in fuel/water test units; viability on subculture; and final pH of the water phase.

JP-5 source	Water phase	Ratings[a] of growth after incubation (weeks) 1	3	7	13	26	Viability[b] after 13 weeks	26 weeks	pH at 26 weeks
Petroleum	SW	-	±	±	+	+	nd	nd	8.0/8.0
	SW + PY	2+	2+	2+	3+	3+	nd	nd	8.5/8.4
Coal	SW	-	±	±	±	-	v	nv	7.85/7.85
	SW + PY	3+	3+	3+	4+	3+	nd	nd	8.5/8.5
Oil shale	SW	-	-	-	+	+	nd	nd	8.0/8.0
	SW + PY	2+	2+	3+	3+	3+	nd	nd	8.4/8.4
75% Petroleum 25% Coal	SW	-	±	±	+	+	nd	nd	7.95/7.95
	SW + PY	2+	2+	3+	3+	3+	nd	nd	8.4/8.25
75% Petroleum 25% Oil shale	SW	-	±	+	2+	2+	nd	nd	7.95/7.95
	SW + PY	2+	3+	3+	4+	4+	nd	nd	8.5/8.3

[a] Rating for Fusarium sp. only: - no growth
± minute amount of growth floating in water phase
+ to 4+ small amount to extensive fluffy growth throughout water phase

[b] See Table 3 for definition of symbols.

Table 11. Growth of P. marina in fuel/water test units; and final pH of water phase[a].

JP-5 source	Water phase	Growth after incubation (weeks)[b] 1	3	7	13	26	pH at 26 weeks
Petroleum	SW	+	+	+	+	+	7.9/8.0
	SW + KH_2PO_4	+	+	+	+	+	5.65/5.7
Coal	SW	±	+	+	+	+	8.05/8.1
	SW + KH_2PO_4	±	±	±	±	±	5.7/5.65
Oil shale	SW	±	±	±	+	+	8.0/8.1
	SW + KH_2PO_4	-	-	-	-	-	6.5/6.55
75% Petroleum 25% Coal	SW	+	+	+	+	+	8.0/8.0
	SW + KH_2PO_4	±	+	+	+	+	5.65/5.7
75% Petroleum 25% Oil shale	SW	+	+	+	+	+	8.0/8.0
	SW + KH_2PO_4	-	-	-	-	-	6.0/6.0

[a] See Table 3 for definition of symbols.

[b] This growth test consists of 0.1 ml of the water phase spread on a marine agar 2216 plate.
- no growth on plate from either duplicate
± sparse growth on plate from at least one duplicate
+ good growth on plates from both duplicates

Table 12. Interfacial microbial growth in two-phase enrichment cultures[a]

Sample No.	Mineral salts overlayed with			Seawater (10%) + PY overlayed with		
	Petroleum	Coal	Shale	Petroleum	Coal	Shale
1-4/21/78	±	+	-	±	+	-
2-4/21/78	2+	-	-	2+	-	-
3-4/21/78	-	-	-	-	-	-
4-4/21/78	-	-	+	-	-	+
5-4/21/78	4+	-	+[b]	+	+	+
6-4/21/78	-	-	±	-	-	-
7-4/21/78	±	-	-	+	-	-
8-4/21/78	3+	+	-	+	+?	-
1-7/26/78	3+	-	-	3+	2+	3+
2-7/26/78	-	-	-	4+	-	-
3-7/26/78	±	-	-	5+	-	-
4-7/26/78	-	+	-	±	-	-
5-7/26/78	-	-	-	2+	-	±
6-7/26/78	3+	3+	+	4+	-	2+
7-7/26/78	2+	2+	-	4+	-	-
8-7/26/78	±	-	-	3+	-	-
9-7/26/78	-	-	-	-	-	-
10-7/26/78	-	-	-	-	-	-
11-7/26/78	-	3+	-	-	4+	-
12-7/26/78	-	-	-	-	-	+
13-7/26/78	+	+	-	3+	+	-
14-7/26/78	4+	4+	+	2+	-	-
M-1-9/2/78	3+	-	2+			
M-2-9/2/78	+	-	-			
M-3-9/2/78	3+	-	2+			
M-4-9/2/78	-	-	-			
M-5-9/2/78	2+	-	-			
M-6-9/2/78	3+	-	+			
M-7-9/3/78	3+	-	3+			
M-8-9/3/78	+	-	2+			
0-1-9/10/78	2+	-	+			
0-2-9/10/78	3+	-	-			
0-3-9/10/78	+	-	+			

[a] See Table 3 for definition of growth ratings.

[b] C. resinae isolated from this culture.